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Modeling maximum lipid productivity of microalgae: Review and next step

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ABSTRACT

Microalgae are fast growing organisms and have the ability to accumulate lipid, which can be converted to biofuels. Here, we review specific growth rate, population productivity, and lipid productivity based on 192 publications of the marine microalgae *Nannochloropsis*. Specific growth rate was reported by thirty publications often using exponential growth equations, and fourteen publications stated biomass productivity. However, direct comparison among productivity estimates is impossible due to differences in calculations or omission of equations. Less than 5% of the publications directly reported lipid productivity, the key parameter for biofuels. We extracted growth data from 30 publications using Plot Digitizer software and tested best fit with exponential and logistic equations. The logistic equation often represents growth data better than the exponential one. Furthermore, we argue that maximum sustainable yield (MSY) is a more useful measure for harvest rates than specific growth rates. Interestingly, MSY displayed closer linear relationships with carrying capacity measures (r^2=0.780, $p<0.001$ and r^2=0.552, $p<0.001$ for algae density and biomass, respectively) than growth rate (r^2=0.297, $p<0.001$ and r^2=0.095, p=0.090). We propose to apply concepts of logistic growth, carrying capacity and MSY calculations to estimates of maximum lipid productivity, similar to commonly used density and biomass calculations.

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Contents

1. Introduction

It is increasingly recognized that global power supplies will have to face a substantial change; away from fossil fuels toward regenerative energy sources. This is due to heightened energy demand, limited supply of fossil fuels and serious environmental challenges caused by anthropogenic greenhouse gas emissions [1]. Electricity generation techniques such as river damming have been over-developed, and all over the world there are over 45,000 large dams with severe impacts on natural river ecosystems [2]. Currently, renewable electricity supplies account only for a minor fraction of global energy demand, and even in future it will probably be impossible to displace fossil fuels entirely [3]. One alternative renewable energy source is biologically produced fuels, which are transportable and thus being touted as the most potential pathways to reduce the dependence on fossil fuels and the emissions of greenhouse gas [4]. Terrestrial plant-derived

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biofuels are controversial due to inefficient land use and food security [5]. Even combining biofuels from traditional crops, waste cooking oil and animal fats would unlikely be able to satisfy the demand for transportation fuels [6].

In this context, biofuels from microalgae have recently re-emerged as a potential to solve future challenges of energy supply and global climate change, and has re-attracted considerable attention from scientific, industrial and governmental sectors [7]. Microalgae are expected to have significant advantages over conventional energy crops [8]. Many species of algae exhibit much higher growth rate, and can accumulate over 50% lipids of dry weight biomass [9]. They are able to double their biomass within 24 h, and have even been claimed to be up to 20 times more productive compared to cultivated terrestrial plant [10]. One of the compelling reasons for using microalgae as biofuel feedstock is to greatly reduce the competition for arable land with crops for human foods, because microalgae can be cultured on non-agricultural area such as arid land, desert, ocean and lakes [11]. The environmental benefits of algal derived biofuels are that their cultivations cannot only absorb pollutants of nutrient-rich wastewater and capture anthropogenic carbon dioxide, but also do not require herbicides or pesticides [12,13].

While numerous studies have extensively reviewed the potential and advantage of using microalgae as biofuel feedstock, there are still many challenges in the development of algal derived biofuels including algal strain selection, biomass cultivation, harvest management and oil extraction [14]. Most of these processes remain in early stages [15], and all efforts to develop algal biofuels need to maximize lipid productivity and reduce production costs as well as energy requirements [16]. Maximizing lipid productivity is of fundamental importance to the success of any algal biotechnology for biofuel production, and one of the biggest challenges is the scaling up from laboratory scale to commercial scale while maintaining high lipid productivity [17]. However, to date there is little consensus on best practices of how to maximize lipid productivity from small-scale studies on algal cultivation [18], and it seems to limit effective scaling up of algae cultivation. Lipid productivity is an apparently easy concept, but it can give rise to misinterpretations if the theoretical bases of this concept are disregarded. Thus, thorough reviews and explanations from the scientific community are critical in order to make commercial algae lipid production viable [7].

The marine microalgae *Nannochloropsis* is known to have relatively high lipid content and interest has increased exponentially in the past 30 years as shown by increasing number of

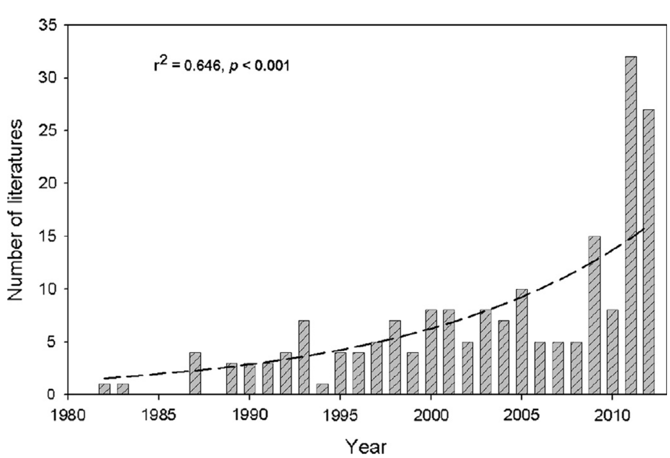


Fig. 1. Publications on the genus *Nannochloropsis* in each year (1982–2012) (Web of knowledge). A total of 192 publications were found.

studies on *Nannochloropsis* (Fig. 1). Since any single study is worth little if not compared and related to others [19], comparisons of a set of studies are at the heart of science and are urgently needed in the development of microalgae biofuels. It is essential that the calculation and report of research data are uniform or can be converted to the same unit among a set of studies to allow quantitative comparisons, such as meta-analysis, which synthesize research findings. Furthermore, it is beneficial when methodology of data collection is consistent and calculations are well established. Originating from pioneering studies in fisheries management in the 1930s, the concept of maximum sustainable yield (MSY) has been well defined by Schaefer (1954), who developed surplus production models for one isolated logistic population under proportional harvesting effort [20]. MSY has been emphasized as the primary goal for sustained harvest management in the fields of fish, wildlife and forestry by almost of international and national plans such as the Implementation Plan (IP, South Africa) [21], Marine Life Management Act (MLMA, USA) [22], Implementing Sustainability in European Union Fisheries (ISEUF, Belgium) [23], Fisheries Management Act (FMA, Australia) [24] and Food and Agriculture Organization of the United Nations (FAOUN) [25]. Like other organisms, microalgae also follow logistic dynamics and experience growth phases including lag, exponential and stationary phase. MSY should be applied to estimate maximum productivity of populations in algal cultivation and sustained harvest management. Here, we took publications of the genus *Nannochloropsis* as a case to present research reviews on specific growth rate, biomass productivity and lipid productivity. In order to calculate maximum productivity of *Nannochloropsis* population based on the concept of MSY, both exponential and logistic equations were employed to test growth curve data (density or biomass over time) which were extracted from these publications. Finally, we propose a concept of "lipid mass population" which is similar to biomass population, and apply the theory of MSY to develop models for maximum lipid productivity.

2. Maximum sustainable yield theory

The review and detailed information of the MSY theory are available in many text books and published literatures. In the following, we only provide a brief introduction of the well known theory as a reference point for the application of the MSY concept to maximum productivity estimations of algal populations. In cases with density-dependent factors, an isolated population generally exhibit logistic growth which can be represented by the two equations:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) \quad (1)$$

and

$$N(t) = \frac{K}{1 + (K - N(0)/N(0))\exp(-rt)} \quad (2)$$

In Eqs. (1) and (2), r is the growth rate of population under ideal conditions (d^{-1}), and K is the population size (density or biomass) when population remains stable and no longer increases and it is called the carrying capacity of the population. In Eq. (1), dN/dt is the rate of change in population size at time t and N is population size at time t; In Eq. (2), $N(t)$ is also population size at time t, and $N(0)$ is the initial population size at the time $t_0 = 0$. When an isolated logistic population is subject to proportional harvesting, we can obtain Schaefer's model [20]:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) - eN \quad (3)$$

In Eq. (3), e is harvesting effort which is dependent on harvesting time and efficiency. The yield (Y) of a population harvested is calculated as:

$$Y = eN \tag{4}$$

when setting an initial condition $N(t=0)=N_0>0$, the solution to Eq. (3) is:

$$N(t) = \frac{AK}{[1+(AK/N_0-1)\exp(-Art)]} \tag{5}$$

In Eq. (5), $A=(1-e/r)$. As long as $e<r$, the solution to Eq. (4) will tend the equilibrium value (N^*):

$$N^* = K\left(1-\frac{e}{r}\right) \tag{6}$$

Then, the equilibrium yield (Y^*) based on Eq. (4) is a quadratic function of e:

$$Y^* = eK\left(1-\frac{e}{r}\right) \text{ or } Y^* = eK - \frac{e^2}{r}K \tag{7}$$

In Eq. (7), when harvesting effort e is optimized to $e_{opt}=r/2$, the maximum sustainable yield (MSY) in the equilibrium is:

$$MSY = \frac{rK}{4} \tag{8}$$

If an isolated population arrives at the stable equilibrium $N^*=K/2$, the MSY in Eq. (8) can be obtained.

3. Specific growth rate and exponential growth principle

Growth aspect of algal population has often been defined by specific growth rate [18], and this parameter has already been reported by 30 publications on the genus *Nannochloropsis* which accounted for 15.6% of all collected (Table 1). Specific growth rates varied dramatically among different literatures, and Sandnes et al. (2005) detected the highest value (1.600) [30], while Banerjee et al. (2011) reported the lowest value (0.004) [34]. Also, five publications defined growth aspect as doubling time (ranging from 0.58 to 50.9 days, Table 1). Doubling time of a population is the time at which it takes the population to double in size, and it can be converted to specific growth rate following the equation:

$$T_d = \frac{\ln(2)}{\mu} \tag{9}$$

In Eq. (9), specific growth rate is usually determined according to the first principle of population ecology. The principle is also called as the Thomas Malthus' exponential law of population growth, stating that population can grow at exponential rates in the presence of abundant resources. It can mathematically be expressed by two famous equations:

$$\frac{dN}{dt} = \mu N \tag{10}$$

and

$$N(t) = N(0)\exp[\mu(t-t_0)] \tag{11}$$

In Eq. (10), the rate μ is called 'growth rate of exponential population' or 'intrinsic rate of increase' and is often considered constant (at its maximal value) when algae culture remain in exponential growth phase [30]. dN/dt is the rate of change in population size over time t and N is population size (measured as density or biomass) at time t; In Eq. (11), $N(t)$ is the population size at time t and $N(0)$ is the initial population size at the time $t_0=0$.

On the basis of Eqs. (10) and (11), specific growth rate of algal population has been estimated in term of algal density by six publications (Table 1):

$$\mu = \frac{\ln(N(t)/N(0))}{t-t_0} \tag{12}$$

In Eq. (12), $N(t)$ is algal density (cells L^{-1}) at the time t; $N(0)$ is initial density (cells L^{-1}) at $t=0$ and t is the cultivation time (d); μ was resulted from a linear fit in a semi-logarithmic plot of algal density against time. In term of algal biomass, specific growth rate of algal population was also determined by six other publications (Table 1):

$$\mu = \frac{\ln(W_t/W_0)}{t-t_0} \tag{13}$$

In Eq. (13), W_t is algal biomass concentration (g L^{-1}) at the time t, W_0 is initial biomass concentration (g L^{-1}) at $t=0$ and t is the experimental time (d); specific growth rate can also be determined from the slope of a semi-log plot of biomass concentration versus time. Algal biomass concentration (W, g L^{-1}) equals cellular weight (CW, g $cell^{-1}$) multiplied by algal density (N, cells L^{-1}). Eq. (13) can then be written as:

$$\mu = \frac{\ln(N(t)\times CW_t)/(N(0)\times CW_0)}{t-t_0} \tag{14}$$

In Eq. (14), CW_t is cellular weight of algae (g $cell^{-1}$) at the time t and CW_0 is initial weight of algal cell (g $cell^{-1}$). Given that cellular weight remains constant during algal growth ($CW_t=CW_0$), it can be expected that two equations for estimating specific growth rate (Eqs. 12 and 13) can be derived from each other and both of them

Table 1
Published data of specific growth and doubling time for the marine microalgae Nannochloropsis.

Groups	References	Species	Specific growth rate (d^{-1})	Doubling time time (days)	Equation
a	[26]	*N. salina*	0.335–0.613		(12)
	[27]	*N. gaditana*	0.108–0.439		(12)
	[28]	*N. salina*	0.260–0.497	1.39	(12)
	[29]	*N.* sp.	0.014–0.278	2.5–50.9	(12)
	[30]	*N. oceanica*	0.600–1.600		(12)
	[31]	*N.* sp.	0.400–0.720		(12)
b	[32]	*N. oculata*	0.078–0.282		(13)
	[33]	*N. salina*	0.07–1.300		(13)
	[34]	*N. oculata*	0.004–0.010		(13)
	[35]	*N.* sp.	0.049–0.447		(13)
	[36]	*N. oculata*	0.194–0.571		(13)
	[37]	*N. limnetica*	0.370–0.870		(13)
c	[9]	*N.* sp.		0.583	
	[38]	*N.* sp.		1.15	
	[39]	*N.* sp.	0.550–0.810		
	[40]	*N.* sp.	0.182–0.505		
	[41]	*N. gaditana*	0.067–0.217		
	[42]	*N. salina*	0.040–0.530		
	[43]	*N. oculata*	0.100–0.130		
	[44]	*N.* sp.	0.17		
	[45]	*N. oculata*	0.060–0.280		
	[46]	*N. oculata*	0.153–0.730		
	[47]	*N. oculata*	0.689–0.862		
	[48]	*N. oculata*	0.155–0.899		
	[49]	*N. gaditana*	0.348–0.565		
	[50]	*N.* sp.	0.165–0.520		
	[51]	*N.* sp.	0.060–0.600		
	[52]	*N.* sp.	0.245–0.319		
	[53]	*N. oculata*	0.013–0.900		
	[54]	*N.* sp.	0.005–0.726		
	[55]	*N. salina*	1.3		
	[56]	*N. salina*	0.72	0.958	

are equivalent to Eq. (14). However, algal cellular weight has been found to exhibit obvious variation among different growth phases [55]. Their finding indicated that specific growth rate estimated from density (Eq. 12) differed from growth rate estimates obtained from biomass (Eq. 13).

Table 2
Published data of biomass productivity.

References	Species	Biomass productivity	Unit
[30]	*N. oceanica*	0.244–0.783	$g\,L^{-1}\,d^{-1}$
[50]	*N.* sp.	0.161–0.239	$g\,L^{-1}\,d^{-1}$
[57]	*N.* sp.	0.110–0.220	$g\,L^{-1}\,d^{-1}$
[58]	*N.* sp.	0.140–0.210	$g\,L^{-1}\,d^{-1}$
[59]	*N.* sp.	0.410–0.760	$g\,L^{-1}\,d^{-1}$
[60]	*N.* sp.	0.228–1.700	$g\,L^{-1}\,d^{-1}$

Table 3
Published data Publications reporting both (lipid content and biomass productivity).

References	Species	Lipid content (%)	Biomass productivity	Unit
[33]	*N. salina*	9.0–34.0	0.002–0.575	$g\,L^{-1}\,d^{-1}$
[36]	*N. oculata*	22.7–41.2	0.296–0.497	$g\,L^{-1}\,d^{-1}$
[61]	*N.* sp.	26.5–42.8	0.400–0.750	$g\,L^{-1}\,d^{-1}$
[62]	*N.* sp.	52.0	46.000	$g\,m^{-2}\,d^{-1}$
[64]	*N.* sp.	32.0–60.0	0.300–0.360	$g\,L^{-1}\,d^{-1}$
[65]	*N.* sp.	33.3–60.7	0.053–0.376	$g\,L^{-1}\,d^{-1}$
[66]	*N.* sp.	14.7–32.5	0.610–1.450	$g\,L^{-1}\,d^{-1}$
[67]	*N.* sp.	55.0	0.330	$g\,L^{-1}\,d^{-1}$

According to Eq. (12) and Eq. (13), we classified the 30 publications with data on specific growth rate into three groups (a–c). Due to the difference between Eqs. (12) and (13), the six studies on specific growth rate from Group a cannot be directly compared to that from Group b. Neither one of the two Eqs. (12 and 13) was clearly identified by the eighteen publications from Group c, accounting for 60% of publications reporting specific growth rate. Since the calculations of specific growth rate are not uniform, the published data from Group c were not used for comparisons. We thus concluded that published data are still not enough to synthesize research findings of the marine microalgae *Nannochloropsis*. We also emphasize that quantitative comparisons of other microalgae have to specify equation used for calculation (e.g., Eqs. (12) or (13)), and future publications should standardize calculations of specific growth rate. As a standard, Eq. (13) will probably be better than Eq. (12), because population biomass can be considered as a more important parameter compared to population density.

4. Population productivity and logistic growth model

The concept of specific growth rate based on exponential growth principle has often been used to advocate that microalgae exhibit much higher productive than cultivated terrestrial plant. The first principle of population ecology (referred to as "first principle" hereafter), represented by the exponential equation (Eqs. 10 or 11), is actually the simplest model of population growth because it assumes that growth rate remains constants. The exponential principle puts an emphasis on population growth

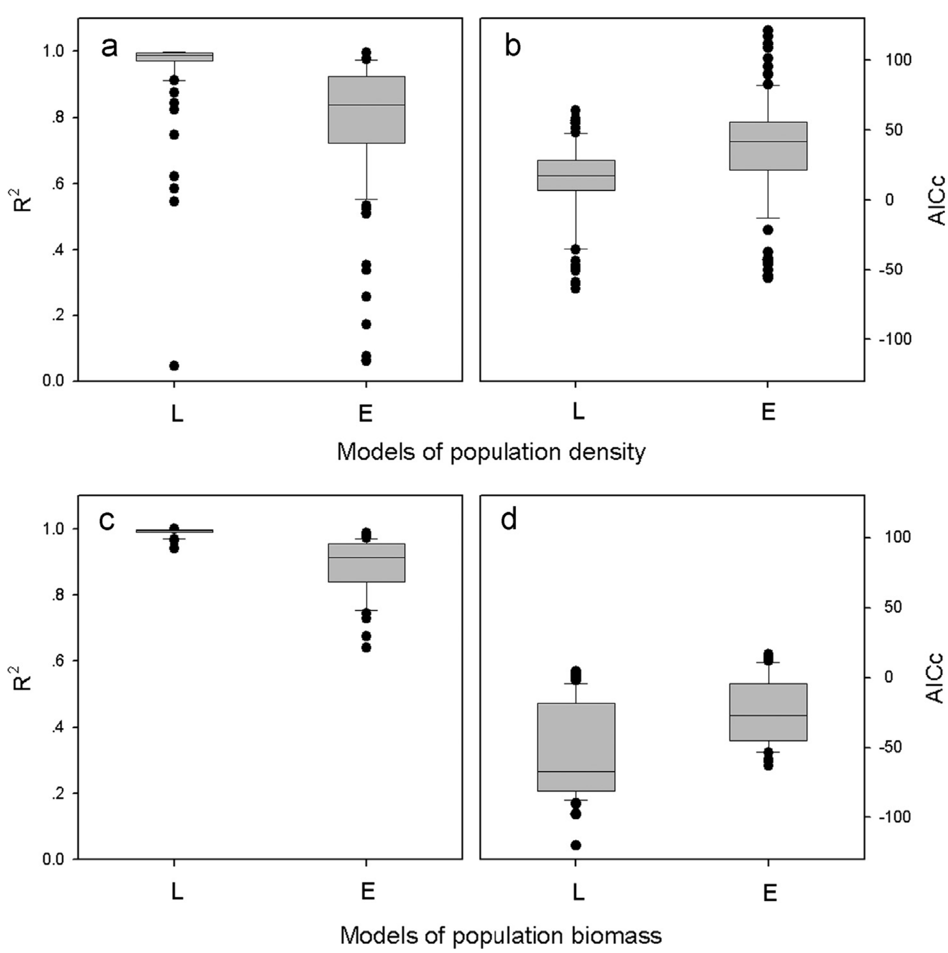


Fig. 2. Performances of logistic (*L*) and exponential (*E*) models for population density (a: R^2, b: AICc) and biomass (c: R^2, d: AICc).

in an unlimited environment, where the effect of increasing density does not need to be considered. Given that the first principle can be applied to determine specific growth rate (μ) as a basic growth parameter for biomass production, it should be expected that algal population show exponential growth in an environment independent of population density dependent effects. However, the second principle of population ecology (from now on referred to as "second principle") which is expressed by Eqs. (1) and (2), demonstrates that exponential growth of any population cannot continue indefinitely and the rate of population increase depends on population size. This principle is also called "self limitation of population increase". Based on the second principle of population ecology, density dependent effects should be considered to better understand microalgae productivity.

Biomass productivity is one of two desirable variables for microalgae lipid productivity, and has been reported by 14 publications on the genus *Nannochloropsis* (Tables 2 and 3). However, these literatures did not provide a detailed description about its equations for estimating biomass productivity. To illustrate how the application of the second principle can provide insights into productivity estimates, by employing exponential (Eq. 11) and logistic (Eq. 2) equations, we modeled data obtained from the literature for population growth and compared performances (goodness of fit) of two modeling approaches (Fig. 2). For our model, Plot Digitizer software (http://plotdigitizer.sourceforge.net/) was used to extract data of population growth from the published curves. We classified the collected curves into two groups: density and biomass, which are used to measure population size. For the marine microalgae *Nannochloropsis*, we extracted 94 density curves from 31 graphs by 20 publications and 48 biomass curves from 11 graphs by 10 publications (Table 4). Using SPSS 13.0 software, we carried out Nonlinear Regression analysis to fit exponential (Eq. 11) and logistic (Eq. 2) equations for population growth. The performances (goodness-of-fit) of these two equations were evaluated using the coefficient of determination (R^2) and Akaike information criterion (AICc). The higher the R^2, the more ideal the model [84], while AICc is a relative measure for the goodness of fit and the model with the lower value provides a better fit to the data [85]. The significant differences of R^2 and AICc between exponential and logistic equations were examined with Paired-Sample *t*-Test. For the 94 density curves, logistic modeling Eq. (2) resulted in significant higher R^2 (Paired-Samples *t* Test: $n=94$, $t=10.5$, $p<0.01$, Fig. 2a) and significant lower AICc ($n=94$, $t=-13.7$, $p<0.01$, Fig. 2b) than exponential modeling (Eq. 11). Similarly, logistic modeling for the 48 biomass curves showed significant greater R^2 ($n=48$, $t=8.8$, $p<0.01$, Fig.2c) and significant smaller AICc ($n=48$, $t=-12.5$, $p<0.01$, Fig. 2d) than exponential modeling. We concluded that logistic modeling for growth of *Nannochloropsis* population has significantly better performances than exponential modeling. We emphasized that the second principle of population growth is a more important scientific basis for modeling microalgae growth than the first principle. Additionally, we demonstrated, through a comprehensive data collection and analyses, the necessity for uniform calculations and reporting of growth rate on microalgae.

Table 4
Sources and summary of density and biomass data for exponential and logistic modeling. 'Fig' refers to the graphs (Figure numbers) that were scanned from each publication. 'Curves' refers to the total number of growth curves from which data were extracted with Plot Digitizer software and used in this study.

	References	Species	Fig	Curves
Density data	[26]	*N. salina*	Fig. 2	2
	[27]	*N. gaditana*	Fig. 1	4
	[28]	*N. salina*	Fig. 2–7	18
	[38]	*N.* sp.	Fig. 6	2
	[41]	*N. gaditana*	Fig. 1	8
	[45]	*N. oculata*	Fig. 1 and 2	9
	[49]	*N. gaditana*	Fig. 8	1
	[53]	*N. oculata*	Fig. 1	2
	[55]	*N. salina*	Fig. 1	1
	[68]	*N. oculata*	Fig. 1	3
	[69]	*N. oculata*	Fig. 3	6
	[70]	*N.* sp.	Fig. 1 and 4	5
	[71]	*N. salina*	Fig. 1	5
	[72]	*N. oculata*	Fig. 1	4
	[73]	*N. gaditana*	Fig. 3	2
	[74]	*N. oculata*	Fig. 1	7
	[75]	*N. oculata*	Fig. 1	4
	[76]	*N.* sp.	Fig. 1–4	9
	[77]	*N.* sp.	Fig. 1	1
	[78]	*N.* sp.	Fig. 1	1
Sum	20			94
Biomass data	[30]	*N. oceanica*	Fig. 8	9
	[32]	*N. oculata*	Fig. 2	5
	[57]	*N.* sp.	Fig. 1	4
	[61]	*N.* sp.	Fig. 1	7
	[62]	*N. oculata*	Fig. 2	3
	[79]	*N. oculata*	Fig. 1	2
	[80]	*N.* sp.	Fig. 1 and 2	5
	[81]	*N.* sp.	Fig. 1	5
	[82]	*N.* sp.	Fig. 1	4
	[83]	*N.* sp.	Fig. 1	4
Sum	10			48

As illustrated in the second section on MSY theory, growth rate (r) and carrying capacity (K) are two key parameters to calculate maximum sustainable yield for microalgae production. We selected logistic equation with R^2 values greater than 0.900 as candidate models for estimating population growth rate (r) and carrying capacity (K). There were 86 and 48 candidate models which accounted for 91.5% and 100.0% of logistic equations for density and biomass, respectively. We employed 'percent relative error' to measure the difference of estimated carrying capacity of logistic models from observed maximum value of experimental curve. The lower the percent relative error, the more precise the model estimates. Models with estimated errors of carrying capacity less than 5% were kept. The present study kept 53 and 31 predicted models which accounted for 61.6 % and 64.6 % of the candidate models for density and biomass, respectively. Estimated carrying capacity (K) and growth rate (r) displayed dramatic variations among the 53 best models of population density, ranging from 8.1×10^6 to 313.9×10^6 cells ml^{-1} and 0.22 to 1.37 day^{-1}. Maximum density carrying capacity (313.9×10^6 cells L^{-1}) and growth rate (1.37 day^{-1}) were estimated by best models for curves published by Roncarati et al. (2004) [72] and Yamasaki and Kawaida (2001) [75], respectively. For the 31 best models of population biomass, there were also clear variations in estimated carrying capacity (K) and growth rate (r) which varied from 0.147 to 6.82 g L^{-1} and 0.17 to 1.88 day^{-1}, and maximum of biomass carrying capacity and growth rate were derived from curves published by Sandnes et al. (2005) [30] and Chen et al. (2012) [62], respectively.

Using carrying capacity and growth rate estimates derived by best models, Eq. (8) was further employed to calculate maximum sustainable yield of density and biomass of *Nannochloropsis* populations. The calculated results showed that MSY values of population density and biomass had dramatic variations, and ranged from 1.3×10^6 to 94.7×10^6 cells mL^{-1} d^{-1} and from 0.02 to 1.18 g L^{-1} d^{-1}, respectively. Maximum of density and biomass MSY were based on curves published by Roncarati et al. (2004) [72] and Chen et al. (2012) [62]. Statistically, MSY values of population density showed a significant linear increase with increasing carrying capacity (Linear regression: $r^2=0.780$, $p<0.01$) and growth rate ($r^2=0.297$, $p<0.01$), but carrying capacity explained much more variance than growth rate (Fig. 3). MSY value of population biomass

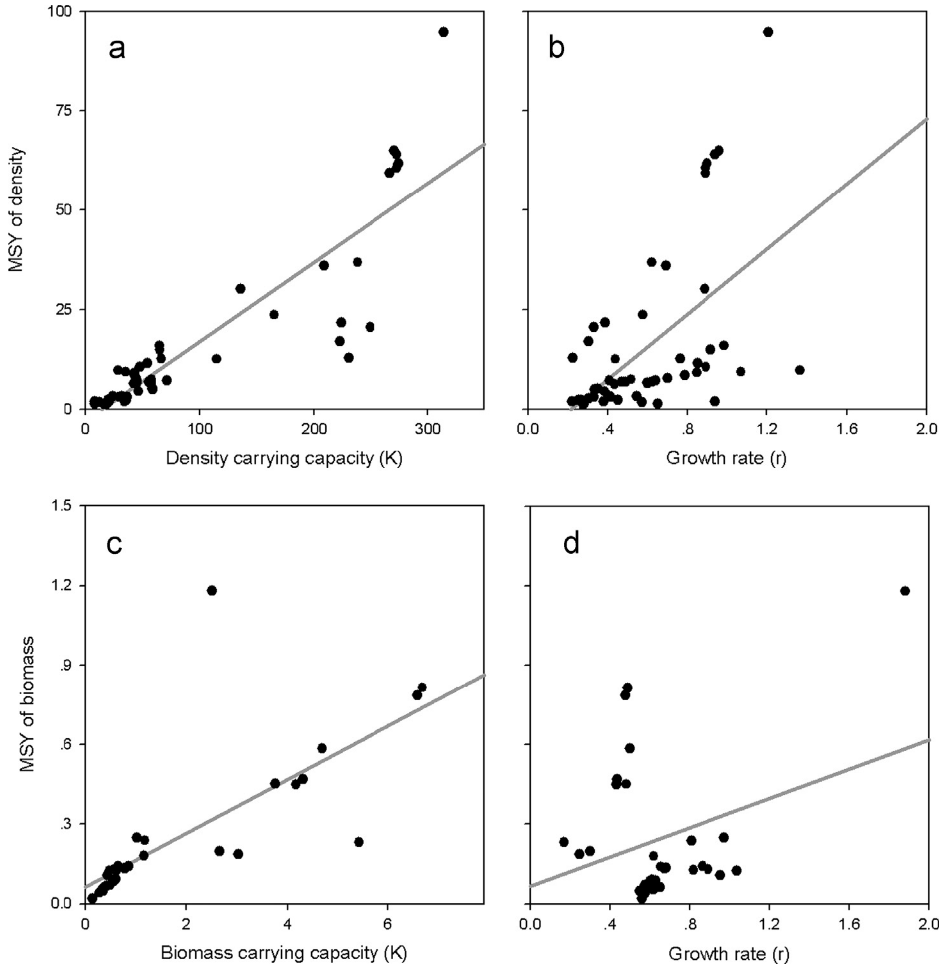


Fig. 3. Linear relationships of MSY of density with algae carrying capacity (a) and growth rate (b) as well as MSY of biomass with carrying capacity (c) and growth rate (d). (a) $r^2=0.780$, $p<0.001$, (b) $r^2=0.297$, $p<0.001$, (c) $r^2=0.552$, $p<0.001$ and (d) $r^2=0.095$, $p<0.090$.

showed significant linear increase with carrying capacity ($r^2=0.553$, $p<0.01$), while it did not statistically related to growth rates ($r^2=0.096$, $p>0.05$) (Fig. 3). These results of regression analysis indicate that maximum productivity of density and biomass are more dependent on carrying capacity than growth rates.

5. Published equations for lipid productivity

Lipid productivity should be quantified in terms of surface area as grams per square meter per day (P_{LA}, g m^{-2} d^{-1}) or on a volumetric basis as grams per liter per day (P_{Lv}, g L^{-1} d^{-1}) [18]. In order to facilitate comparison, research data of lipid productivity have to be consistently presented as a standard unit. Given that the depth (D, m) of algal cultivation system is reported, both units of lipid productivity can be converted to each other by the following formula:

$$P_{LA}=P_{LV}\times D\times 10^3 \qquad (15)$$

Following a flow chart procedure (Fig. 4), the 192 publications on the genus *Nannochloropsis* can be classified into five groups (Group A–E). Group A was comprised of eight publications which directly presented lipid productivity, but this group accounted for less than 5% of all collected literatures. In term of surface area, lipid productivity of *N. salina* has been measured at 4.0 g m^{-2} d^{-1} only by one early experiment [56]. A recent publication of *N. oculata* showed that this species stands out as having high lipid productivity, with a record of 0.324 g L^{-1} d^{-1} (Table 5) on a volumetric basis. Because the literatures of Group A did not report the depth for algal cultivation system, the conversion between units of measurement cannot be carried out by Eq. (15) to compare lipid productivity of recent records to an early finding. It should thus be emphasized that the reports of cultivation system depth are necessary for the conversion and comparison of lipid productivity data. Group B included three literatures which described productivity of eicosapentaenoic acids (EPA), with a record of 0.011 g L^{-1} d^{-1}. The productivity of total fatty acids (TFA) was reported by Group C with only one literature, ranging from 0.132 to 0.400 g L^{-1} d^{-1}, while hydrocarbon (HC) productivity was measured by one literature of Group D, with the range from 0.055 to 0.070 g L^{-1} d^{-1}. The concept of lipid is obviously different from that of EPA, TFA and HC, and the published data from Group B–D are not reflecting lipid productivity and cannot directly be compared to data of Group A. In Group E, there were over 90% of the collected literatures, which did not present EPA, TFA, HC productivity nor lipid productivity. Despite high expenses of lipid analyses, lipid productivity is a key parameter for biodiesel production, and it is essential, that future research focuses on lipid productivity and reports results in standard units.

The published data of lipid productivity available so far are insufficient for data-sharing, decision-making and scaling up of laboratory conclusions to commercial scale, especially since the publications did not provide a detailed description on the estimated methods of lipid productivity. Even within Group A, which reports lipid productivity, we only found two publications describing equations used to estimate lipid productivity [43,79]. Su et al.

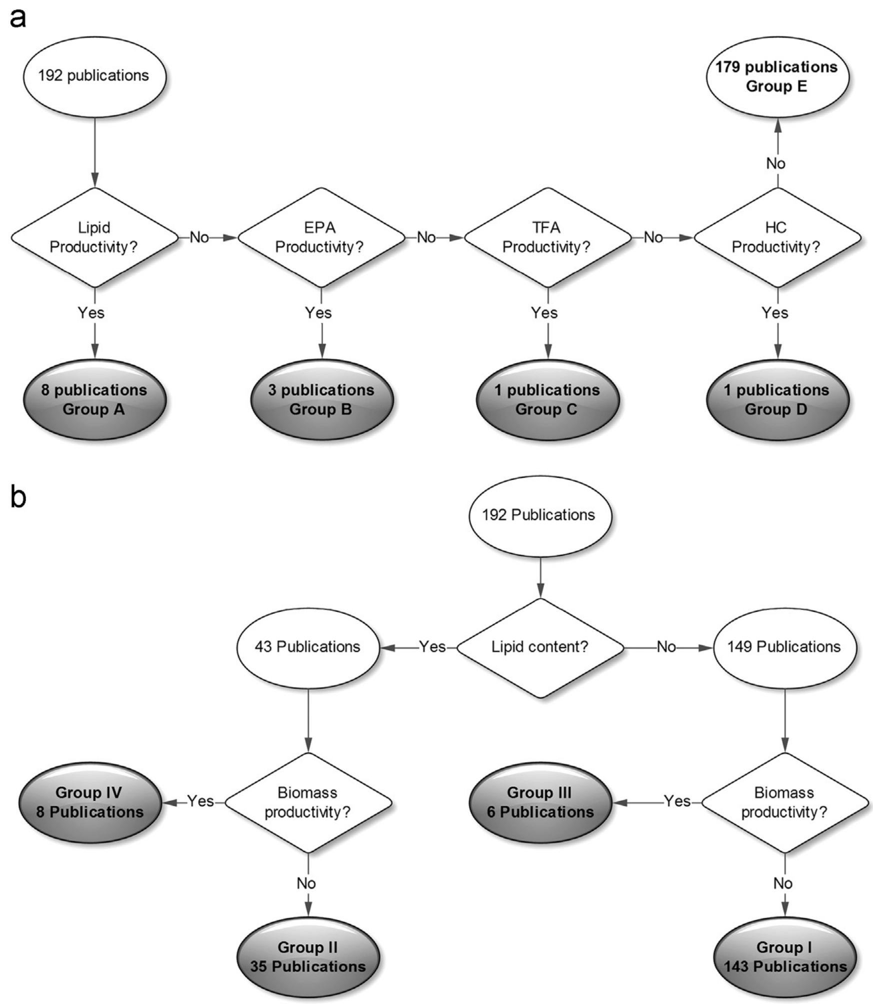


Fig. 4. Simple flow chart for classifying the publications of the genus *Nannochloropsis* (a) following lipid productivity and (b) following lipid content and biomass productivity. See Table 5 for a more detailed listing of publications that are represented in first flow chart (a). See Table 6 for citations of Group II, Table 2 for citations of Group III, and Table 3 for citations of Group IV that are presented in second flow chart (b).

(2011) proposed an estimated method as [79]:

$$P_{LV} = \frac{C_f \times DCW_f - C_i \times DCW_i}{T} \quad (16)$$

In Eq. (16), P_{LV} is lipid productivity in the unit of grams per liter per day (g L^{-1} d^{-1}), and T is the cultivation time (d); C_f and C_i are the final and initial content (%) of algal lipid during cultivation time, and DCW_f and DCW_i are the final and initial biomass concentration (g L^{-1}) of microalgae, respectively. The product of C_f and DCW_f equals the final concentration of lipid mass (LM_f, g L^{-1}), and that of C_i and DCW_i equals the initial concentration of lipid mass (LM_i, g L^{-1}). The initial concentration of lipid mass can be shown to be negligibly small, and the value has been neglected in the equation of Converti et al. (2009) [43]:

$$P_{LV} = \frac{LM_f}{T} \quad (17)$$

Eq. (17) can be considered equivalent to Eq. (16) employed by Su et al. (2011) [79]. Lipid productivity in the experiment of Su et al. (2011) [79] ranging from 0.103 to 0.324 g L^{-1} d^{-1} was up to 20 times greater than that of Converti et al. (2009) [43] with a range from 0.009 to 0.016 g L^{-1} d^{-1}, although they cultured the same marine algae species (*N. oculata*). The differences of lipid productivity depend largely on their cultivation time (T), with 4 and 14 days from the studies of Su et al. (2011) [79] and Converti et al. (2009) [43], respectively. The cultivation time should thus be paid great attention to, when utilizing the research conclusions of lipid productivity which resulted from Eqs. (10) or (11). It also seems that lipid productivity calculated from Eqs. (16) or (17) can be considered constant during cultivation time. Since microalgae can be seen as isolated logistic population within its cultivation

Table 5
Published data of lipid productivity for marine algae *Nannochloropsis*. Group A=total lipid productivity, Group B=eicosapentaenoic acids (EPA) productivity, Group C=total fatty acids (TFA) productivity and Group D=hydrocarbon (HC) productivity.

Groups	References	Species	Productivity	Units
A	[36]	*N. oculata*	0.084–0.151	g L^{-1} d^{-1}
	[43]	*N. oculata*	0.009–0.016	g L^{-1} d^{-1}
	[56]	*N. salina*	4	g m^{-2} d^{-1}
	[59]	*N.* sp.	0.019–0.032	g L^{-1} d^{-1}
	[61]	*N.* sp.	0.112–0.280	g L^{-1} d^{-1}
	[64]	*N.* sp.	0.117–0.204	g L^{-1} d^{-1}
	[79]	*N. oculata*	0.103–0.324	g L^{-1} d^{-1}
	[86]	*N.* sp.	0.000–0.012	g L^{-1} d^{-1}
B	[31]	*N.* sp.	0.003–0.005	g L^{-1} d^{-1}
	[50]	*N.* sp.	0.007–0.011	g L^{-1} d^{-1}
	[60]	*N.* sp.	0.001–0.003	g L^{-1} d^{-1}
C	[39]	*N.* sp.	0.132–0.400	g L^{-1} d^{-1}
D	[63]	*N.* sp.	0.055–0.070	g L^{-1} d^{-1}

system, Eqs. (16) or (17) can only be employed to estimate average lipid productivity rather than maximum sustainable yield of lipid mass during cultivation time. Thus, the equations for maximum lipid productivity need to be developed according to the law of logistic growth.

Because few publications directly reported lipid productivity, it has only been calculated as the product of lipid content and biomass productivity using the following equation (18):

$$P_{LV} = C \times P_{BV} \tag{18}$$

In Eq. (18), lipid content (C) was reported as percentage of algal dry weight (%) and biomass productivity (P_{BV}) in grams per liter per day ($g\ L^{-1}\ d^{-1}$). According to the two independent variables of Eq. (18), the 192 publication of the genus *Nannochloropsis* can be re-classified into four groups (Group I–IV, Fig. 5). Neither of the two variables was reported by 136 publications of Group I, which accounted for 73.5% of all publications collected. Group II was comprised of 35 publications, where data were presented as lipid content but biomass productivity was not regarded (Table 6). Lipid content exhibited dramatic variations among different publications, and the highest content (68.5%) was detected by Sforza et al. (2011) [28], while the lowest content (7.0%) was found by the experiment of Hu and Gao (2003) [83]. These variations indicated that lipid content is particularly sensitive to cultivation conditions such as light, temperature, salinity and nutrients. Group III included 6 publications where data were given as biomass productivity without reporting lipid content (Table 2). Biomass productivity also showed significant variations among different literatures, due to the strong influence of cultivation conditions. When using lipid content of Group II and biomass productivity of Group III, two independent variables of Eq. (18) were subjected to different cultivation conditions and thus lipid productivity is likely underestimated or overestimated. Collecting the data of lipid content and biomass productivity from the same cultivation system is important to accurately calculate lipid productivity. Both independent variables were presented together within the literature of Group IV, but this group was comprised of only eight publications and accounted for less than 5% of all collected (Table 3).

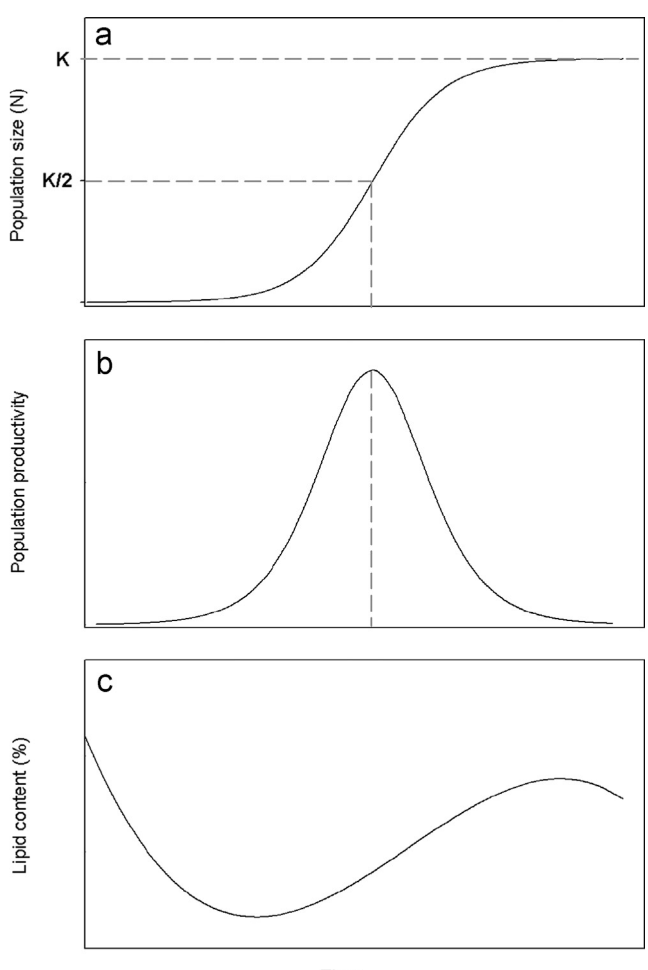


Fig. 5. Graphs representing modeling results for of logistic growth (a), population productivity (b) and corresponding lipid content (c).

6. Optimal models for maximum lipid productivity

Lipid productivity is the lipid mass that can be produced per day and is dependent upon biomass productivity of microalgae and lipid content of their biomass [6]. Maximum lipid productivity will thus be limited not only by maximum biomass productivity, but also by maximum lipid content. Population productivity increases with population size (N) when N is lower than half of carrying capacity (K), and it reaches maximum at $N = K/2$ (Fig. 6a, b); When N is higher than $K/2$, population productivity declines gradually, and becomes zero at $N = K$. Population productivity is thus the quadratic function of cultivation time, and the graph of a quadratic function is the parabola which opens downward (Fig. 6b). Lipid content also displays dramatic variations during population logistic dynamics [55]. Modeling data of lipid content over time, which was exacted from the publication of Emdadi and Berland (1989) [55], indicated that lipid content was a cubic function of cultivation time (Fig. 6c). As demonstrated by the mode graphs, when population productivity reaches its maximum,

Table 6
Published data of lipid content.

References	Species	Lipid content (%)
[9]	*N.* sp.	60
[26]	*N. salina*	11.0–35.8
[27]	*N. gaditana*	1.0–3.0
[28]	*N. salina*	68.5
[32]	*N. oculata*	9.8–14.1
[34]	*N. oculata*	12.5–13.0
[39]	*N.* sp.	13.5–47.7
[42]	*N. salina*	12.0–70.0
[43]	*N. oculata*	7.9–14.9
[46]	*N. oculata*	63.4–77.6
[48]	*N. oculata*	63.4–76.2
[51]	*N.* sp.	13.9–21.7
[55]	*N. salina*	22.8–55.9
[56]	*N. salina*	10.0–25.0
[69]	*N. oculata*	17.2–18.4
[77]	*N.* sp.	8.8–14.9
[79]	*N. oculata*	22.5–44.5
[80]	*N.* sp.	27
[81]	*N.* sp.	32.1–42.7
[83]	*N.* sp.	7.0–9.0
[86]	*N.* sp.	9.0–62.0
[87]	*N.* sp.	40.0–43.5
[88]	*N. oculata*	11.0–30.0
[89]	*N. salina*	20.0–50.0
[90]	*N. oceanica*	15.0–20.0
[91]	*N. oculata*	3.6–4.3
[92]	*N.* sp.	45.8
[93]	*N.* sp.	20.8–55.7
[94]	*N. salina*	40
[95]	*N.* sp.	15.0–45.0
[96]	*N.* sp.	28.0–43.0
[97]	*N. gaditana*	18.6–35.3
[98]	*N. oculata*	21.4
[99]	*N. sp.*	25.5
[100]	*N. oculata*	30.7–41.4

lipid content keeps increasing; when lipid content increases to its maximum, population productivity becomes zero. It is thus impossible that an isolated population synchronously reach maximum biomass productivity and maximum lipid content during logistic dynamics. Maximum lipid productivity cannot be estimated as the product of maximum biomass productivity and maximum lipid content, and the most important next step is to develop optimal models for maximum lipid productivity.

Biomass of microalgae population can be estimated as the product of population density and cellular biomass. Although cellular biomass of microalgae displays obvious variations during population growth, population biomass has been well used to measure population size and replace population density in logistic growth models (Eqs. 1 and 2). Lipid-mass of population can be defined as the product of population density and cellular lipid mass. Like a replacement of population density by population biomass, we propose a change of logistic model parameter from population density into population lipid-mass. Thus, the logistic equation of population lipid-mass can be written as:

$$M_L(t) = \frac{K_L}{1+((K_L - M_L(0))/M_L(0))\exp(-r_L t)} \tag{19}$$

In Eq. (19), r_L is the growth rate of population lipid-mass under ideal conditions (d^{-1}), and K_L is interpreted as carrying capacity of population lipid-mass. $M_L(t)$ is population lipid-mass at time t, and $M_L(0)$ the initial lipid-mass of population at the time $t_0 = 0$. Like maximum sustainable yield of population biomass, when harvesting effort (e) of population lipid-mass is optimized to $e_{\text{opt}} = r_L/2$, MSY of population lipid-mass in the equilibrium is:

$$MSY_L = \frac{r_L K_L}{4} \tag{20}$$

When the population arrives at the stable equilibrium of lipid-mass $M_L^* = K_L/2$, MSY_L in Eq. (8) will be obtained.

7. Concluding remarks

Potentially, microalgae cultivation brings about a highly attractive and ecologically-friendly generation of biofuels. However, we need to recognize that theoretical and technical problems still have to be overcome, before algae biofuels will become a reality [4]. Understanding theoretical knowledge of microalgae cultivation will certainly play a key role in the technical development of algal derived biofuels. Scientifically, the exponential and logistic laws of population growths have been recognized as key theories in population ecology and are also called the first and second principles, respectively. In the present review of the marine microalgae *Nannochloropsis*, we applied the principles of population ecology to evaluate and understand the concepts and calculations of specific growth rates and population productivity. Originating from exponential growth, specific growth rates have been used to claim the potential advantage of microalgae over terrestrial plant. Our comparisons of modeling results showed that exponential models had significantly lower performances than logistic models. We thus argued that the concept of specific growth rate is not suitable for the evaluation of microalgae growth advantage, and that the theoretical basis for microalgae cultivation has to be changed from the first principle to the second principle of population ecology. Biomass cultivation of microalgae needs to be evaluated by maximum population productivity (MSY), which is the product of carrying capacity and growth rate under the logistic model. Our regression analysis found that maximum population productivity is more dependent on carrying capacity than growth rate. Compared to biomass cultivation of microalgae, lipid-mass cultivation seems more attractive to biofuel feedstock and lipid productivity is a more useful concept than population productivity. Most publications focused on either population growth or lipid content, and few publications directly reported lipid productivity and carefully described the evaluated methods. Following the modeling process of maximum biomass productivity, we replaced population biomass of logistic models with population lipid-mass to and proposed the concept of maximum lipid productivity and its optimal models.

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